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A rapid, automated gradient flow injection–spectrophotometric technique for study of metal complexation reactions

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ABSTRACT

Controlled dispersion as generated in flow injection analysis (FIA) essentially permits an infinite variety of known compositional gradients. Using this unique advantage of FIA, the stability constants of metal complexation are calculated by injecting an aliquot of metal solution into the flow of ligand solution in a single-line manifold. While the ligand dilution is negligible, the concentration gradient of injected metal ion can be calculated from the dispersion pattern which is calibrated previously using a dye solution. To show the simplicity, versatility and ease of instrumental setup over approaches based on the classical titration, the method was applied to determine stability constants of murexide with several metal ions. The SQUAD computer program was used for fitting the predefined complexation model to the spectral-mole ratio data. The proper selection of the chemical model was verified by the determination of the number of absorbing species by using a singular value decomposition of each data set. The stability constants obtained for murexide and metals including Cu^{2+},Cd^{2+}, Pb^{2+}, Ca^{2+} and Co^{2+} are 4.35, 4.27, 4.50, 2.55 and 2.57, respectively. The formation constants determined here are in good agreement with those previously reported and with those obtained from conventional batch titrations. The main advantage over the classical batch titration method is that by utilizing just one injection per sample, the proposed method reduces experimental error by reducing the experimental steps needed to obtain the required spectral-mole ratio data. The details of the proposed method are discussed.

1. Introduction

The use of stability constants of metal complex formation as an effective measure of affinity of a ligand for metal ion in solution have a long history, and are an excellent and quantitative index for the success or failure of ligand design [1,2]. In three recent decades, three important developments in the solution coordination chemistry provide a major role for stability constant determinations and further developments of the field [3]. These are, first, the development of the chemistry of macrocyclic and macrobiocyclic complexes [4], with the accompanying opportunities to ligand design and synthesis of new ligands with novel properties and applications; second, the develpomet of the new fields in bioinorganic and inorganic envirmonmental chemistry [5–7], which require a careful study of the complexes formed in multicomponent systems containing more than one ligand and one metal ion; and at last, the development of computers and computer programs for processing equilibrium data to provide rapid determination of stability constants with higher accuracy, and the potential for studying multidentate ligands and systems of many metal ions and ligands, that are too complex to have been investigated by classical methods [8–13]. Nowadays, the calculated stability constants using computer programs, are used for the elucidation of molecular and ionic species present in complex biological and enviromental systems [5].

Among the various methods for studying the complexation equilibria in solution, manipulation of spectrophotometric data with computer programs is a powerful method under extensive experimental conditions [14]. Generally, spectrophotometric methods are highly sensitive and suitable for studying chemical equilibria in solutions. When the spectral profiles of the components involved in the chemical equilibrium are distinct and not overlapped, their concentrations can be measured directly, and the calculation of equilibrium constants can be made with a blink an eye. However, in many cases, the spectral profiles of components are overlapped and analysis is not straightforward [15]. There are several advantages of using multiwavelength data (as compared to selecting single wavelengths) including [16] (a) appropriate analysis results in determination of the pure spectra and concentration profiles for all reacting species. (b) Multivariate data allow the application of a wide range of model free analyses. (c) The need to determine a “good” wavelength to follow the reaction is eliminated. (d) The analysis of multiwavelength data is often significantly more robust. The disadvantages of multiwavelength data

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include the large number of data that are acquired in a short time and the large number of parameters that need to be fitted. Readily available personal computers with large memory solve the first problem and appropriate algorithms solve the second. Keeping the above mentioned advantages, there are several reports in literature trying to use multivariate approaches in determination of stability constants [17–22] and hence, there are monographs for calculation of stability constants from multivariate data [23,24].

In the conventional methods of complexometric titrations, the preparation of a series of mixtures, with constant concentration of one of the components (usually ligand) and varying concentrations of the other (usually metal), is required. These mixtures may be prepared and measured in a batch mode or a manual stepwise titration mode [25–27] and the complete study of the complexation phenomenon requires a wide range of concentration ratios and manipulation is necessary in each step of titration.

Flow analysis has experienced amazing developments in recent decades and continues to evolve [28]. Developments of FIA have been further stimulated by the additional advantages of automation, such as increased precision, decreased cost of individual assay, and the satisfactory reliability of automated equipment [29]. One of the important features of the flow injection method is the possibility of adapting the flow pattern to the requirements of a particular determination [30] and hence, continuous flow titration methods have been reported by many investigators [31–40].

In the present work, we wish to report the utility of FIA in conjunction with a photodiode array UV–vis spectrophotometer and a whole-domain spectral processing computer program, SQUAD, in the study of complexation reactions between murexide and some transition metal ions. The unique and reproducible controlled dispersion aspect of FIA [32] is used for generation of required absorption spectral-mole ratio data to follow the complexation reactions. In the conventional techniques for determination of stability constants, manipulation in every step of titration is mandatory, but in the current method only three and one injections are required before and after dispersion calibration of the reactants, respectively. Other advantages include automation and absolutely no manipulation, skillfulness and carefulness during the titration. By injection of a desired volume of a metal ion solution with proper concentration into flow of ligand, all of the required absorption spectral-mole ratio data are collected to determine the stability constants of the resulting metal:ligand complexes.

2. Experimental section

2.1. Material and chemical reagents

All reagents used were of analytical grade. Murexide, acetic acid (glacial) and sodium acetate were purchased from Merck. Cu $(NO_3)_2 \cdot 3H_2O$, Cd $(NO_3)_2 \cdot 4H_2O$, Pb $(NO_3)_2$, fluorescein, Co $(ClO_4)_2 \cdot 4H_2O$ and Ca $(NO_3)_2 \cdot 4H_2O$ were purchased from Fluka. The stock 1.00×10^{-1} mol L^{-1} acetic acid/acetate buffer solution was made in distilled water and was further diluted to 1.00×10^{-2} mol L^{-1} for use in the preparation of metal and ligand solution. The 1.00×10^{-1} mol L^{-1} stock solutions of Ca^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+} and Pb^{2+} were prepared in 1.00×10^{-2} M acetate buffer. The fresh solutions of murexide were prepared daily in the 1.00×10^{-2} mol L^{-1} of acetate buffer. The 1.00×10^{-4} mol L^{-1} standard stock solutions of fluorescein sodium salt were made in 1.00×10^{-2} mol L^{-1} acetate buffer for further dilutions.

2.2. Apparatus, instrumentation and software

The FIA manifold used in all experiments is shown schematically in Fig. 1 and the inset of Fig. 1 shows the scheme of the flow

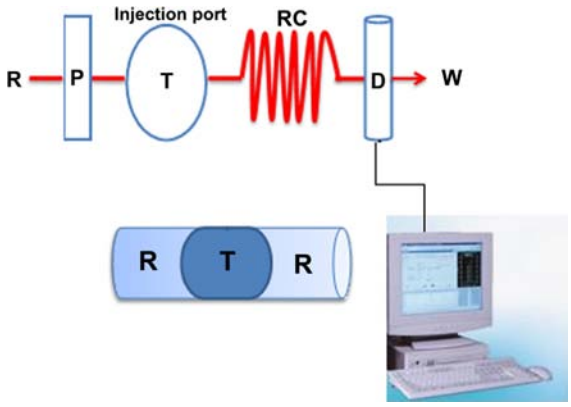

Fig. 1. FIA manifold used for complexometric titration. *R*=Reagent stream (ligand solution in 0.01 mol L^{-1} Acetate buffer), pH=4.72, pumped at 0.75 ml/min rate; *P*=peristaltic pump, *T*=Titrant (Metal in 0.01 mol L^{-1} acetate solution, Injection volume=50 μl); RC=Reaction coil (250.0 cm); *D*=Photodiode array spectrophotometer. *W*=Waste. The inset shows the scheme of the flow segments of titrant and reagent (ligand).

segments of sample and reagent. All tubes employed were silicon (0.80 mm internal diameter and 250.0 cm length). Injection is carried out by using a six-way Rheodyne injection port. In the dispersion calibration step, the carrier stream was a 0.01 mol L^{-1} acetate buffer with a pH of 4.76 and the injected reagent was a murexide solution prepared in the same acetate buffer. In the titration step, carrier stream was a ligand solution prepared in the acetate buffer and the injected sample was a metal solution prepared in the same acetate buffer. For evaluating the dilution effect of metal solution injection into carrier stream of ligand, a blank solution (acetate buffer solution) injected into carrier stream of ligand. A multi-channel Heidolph peristaltic pump (Heidolph PD 5001) was used. The absorption spectra with 1 nm spectral bandpass were recorded using an Agilent-8453 UV–vis diode-array spectrophotometer. Agilent UV–Visible Chem-Station software for data acquisition was used throughout. A 10 μl flow cell with 2.0 mm path length was used. All absorption spectra were recorded in the wavelength range 350–650 nm. The absorption spectra recorded after a single injection of 50 μl of desired solution into carrier stream (both solutions prepared in 1.00×10^{-2} mol L^{-1} acetate buffer solution at pH=4.72). The spectrophotometer starts scanning 200 s after injection, and continues scanning every 6 s for the next 500 s assuring the injected metal solution passes completely through the flow cell. The injected metal passes through the flow cell in the range of 250–655 s and the range analytically adequate for formation constants calculations was in the range of 420–590 s. The pH values were measured and adjusted by an AMTAST pH-meter equipped with combined Ag/AgCl electrode. Home-written Excel-VBA macro was used for preparing required input file format by SQUAD computer program. Further data treatments were done using MATLAB ver. 7.11 [41].

3. Results and discussion

3.1. Theory

Concentration profiles of contributing species in a successive complexation system are calculated by solving a polynomial equation:

$$[L]^{n+1}\beta_n + [L]^n\{\beta_n(nC_{t,M} - C_{t,L}) + \beta_{n-1}\} + [L]^{n-1}\{\beta_{n-1}((n-1)C_{t,M} - C_{t,L}) + \beta_{n-1}\} + \ldots - C_{t,L} \quad (1)$$

where n is the maximum number of successive mononuclear complexes, β_1, β_2,..., β_n are overall formation constants, $C_{t,M}$ and

$C_{t,L}$ are total metal and ligand concentrations at time t after sample injection, respectively and $\beta_0=1$. This polynomial equation results from equations of overall formation constants and mass-balance equations of metal and ligand. The equilibrium concentration of free ligand [L], in each step of complexation reaction (titration step or time step in the current study) can be calculated by resolving polynomial equation (Eq. 1) for known values of n, $C_{t,M}$ and $C_{t,L}$. After calculation of [L], equilibrium concentration of other species, such as [M] and [ML_n], in each step of reaction can be computed [42].

Computer programs have revolutionized the calculation of stability constants, so that, nowadays, very complex systems may be handled with relative ease. The Stability Quotients from Absorbance Data (SQUAD) program was written in FORTRAN by Leggett et al. [13,24,43], and they for the first time used a factor analysis method for determination of stability constants [44]. SQUAD uses non-linear least-squares for calculation of stability constants. As in the linear case, in non-linear least squares, the sum of squares (SSb) of the residual differences between the experimental value and the value predicted by the model is minimized.

$$SS(b)=\sum_{i=1}^{n}\{y_i-\hat{y}_i(b)\}^2 \quad (2)$$

where $\hat{y}_i(b)$ is the estimated value of the response using the non-linear equation with the estimate values b for the parameters β and y_i is the experimental value of the response. The sum runs over all n experimental data points. The least squares estimate "b" of β is those values of the parameters that minimize $SS(b)$ [45].

3.2. Stoichiometry of complexation

Knowing the number of light-absorbing species is essential for subsequent quantitative and qualitative solution equilibrium studies. The number of light absorbing species is estimated using principal component analysis [17,44]. Fig. 2 shows the log singular values calculated by singular value decomposition (SVD) of the data matrix $\mathbf{X}_{n\times p}$ (where n is number of time steps and p number of wavelengths) obtained from FIA dispersion pattern of complexation reaction of murexide and Cu^{2+} as a function of number of components. The inset of Fig. 2 shows the loading plot for the third component. The loading plots of the first two components have meaningful abstract trends and the third one shows no systematic variation which represents the existence of two light-absorbing species. Obviously both representations confirm the presence of the two light-absorbing species that are L and ML in the current study. It is not worthy to mention the M species that has no significant absorbance in the applied wavelength region. The SVD results of other data matrices resulting from the reaction of murexide and metals including Co^{2+}, Ca^{2+}, Cd^{2+} and Pb^{2+}showed the same results and confirmed the formation of only 1:1 complexes.

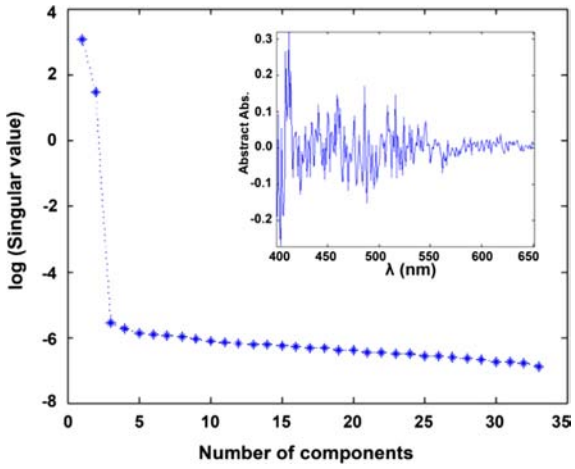

Fig. 2. Logarithmic scale of singular values versus number of components obtained from SVD of murexide–Cu^{2+}data matrix. Inset shows the loading–loading plot for third component.

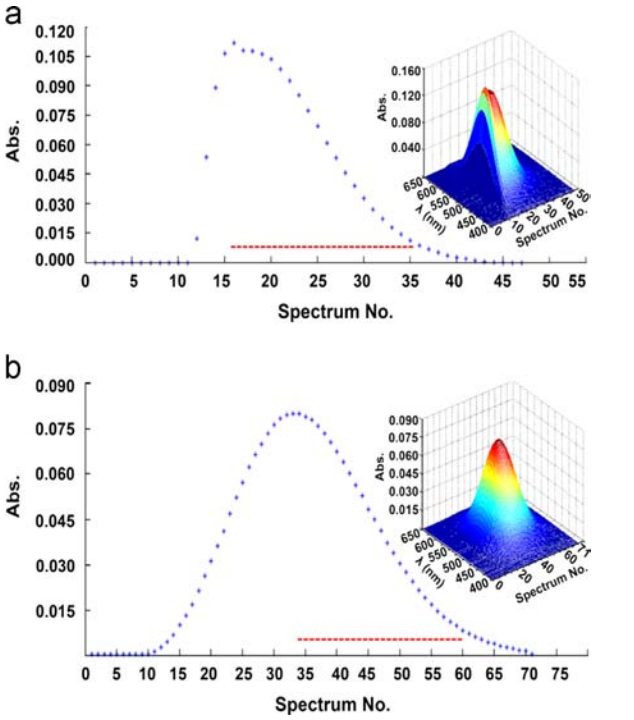

Fig. 3. Dispersion pattern of injected calibrator dye (murexide) into blank solution. (a) Tube length: 50 cm, flow rate: 1.25 ml/min, injection volume: 50 μl, murexide concentration: 4.0×10^{-4} mol L^{-1}, time interval of spectra: 1 S. (b) Reaction coil length: 250 cm, flow rate: 0.75 ml/min, injection volume: 50 μl, murexide concentration: 9.0×10^{-4} mol L^{-1}, time interval of spectra: 6 S. The insets show the corresponding spectra (in three dimensions). The dashed lines show the region used for calculation of dispersion coefficients.

3.3. Metal concentration gradient (C_M) calculation

In order to calibrate dispersion pattern, a dye (murexide or calibrator solution) prepared in the appropriate buffer (0.01 mol L^{-1} HOAC/OAC^-) is injected into the stream of buffer solution (0.01 mol L^{-1} HOAC/OAC^-). The dispersion pattern of injected calibrator solution via reaction coil is given in Fig. 3a and b. The insets of Fig. 3a and b show whole spectra (in three dimensions) that have been used to derive these patterns. Dispersion pattern is resulted from tracking the changes in absorbance of calibrator dye at its maximum wavelength (λ_{max}) versus time after injection. Injection of fluorescein solution into the buffer solution, gives exactly the same dispersion pattern as Fig. 3a and b, this phenomena indicate that dispersion pattern is independent of nature of the injected solution. Dispersion coefficients, D_t, are calculated from Eq. (3)

$$D_t=A_{o,cal}/A_{t,cal} \quad (3)$$

where, $A_{o,cal}$ is the absorbance of injected (calibrator) dye at λ_{max} when its role is as carrier in flow manifold (Fig. 1) and $A_{t,cal}$ is the absorbance of dye at λ_{max} of the dye at time t after injection in the case that the dye solution is injected into carrier stream. The descending part of the dispersion pattern, shown by short dash lines in Fig. 3, was used for calculation of dispersion coefficients and therefore for further calculations, because in the descending

part the changes in concentration along the tube are more monotonic and give more precise and reproducible results.

The calculated dispersion coefficients are typically inside the range of 38–1181 as shown in Table 1. The corresponding RSD values of calculated dispersion coefficients obtained by 5 repeated and subsequent injection of calibrator dye are shown in Table 1. The $D_{t,M}$=38.19 shows the point with minimum dispersion of the injected solution, therefore the concentration of injected compound has the maximum value (the point at the beginning of the dash line in Fig. 3a), but the $D_{t,M}$=1181 at the end of dash line in Fig. 3b, shows the point where almost all of the dispersed injected solution has passed through the flow cell. Since the flow just plays a transferring role [46] and remembering the controlled dispersion aspect of FIA, the calculated dispersion coefficients can be used for determining the concentration gradient of metal at any time step after injection of metal solution ($C_{t,M}$) as Eq. (4)

$$C_{t,M} = C_{0,M}/D_t \quad (4)$$

where, $C_{0,M}$ is the concentration of the injected metal solution and $C_{t,M}$ is the concentration of metal at time t after metal solution injection into ligand solution.

3.4. Adjusting the parameters affecting dispersion pattern

Since C_M is calculated from a predetermined D_t values (Eq. 4), it is essential to assure that the dispersion pattern calibration (performed using a calibrator dye solution) is valid during the complexation titration. A measure for studying the applicability of the suggested method is considering the differences in molecular diffusion coefficients between the calibrator and the metal ion. In a single line FI system (Fig. 1), small variations in diffusion coefficients, change the peak profiles [47] and based on this property, diffusion coefficients [48,49], molecular weights [50] and viscosities [48] have been determined. Based on the Wilke–Chang formula [51] diffusion coefficient is inversely proportional to viscosity and proportional to the square root of the relative mass (M_r). As the molecular weight of the calibrator dye, murexide, and the metal salts do not differ considerably, no significant effect is expected. This is demonstrated by totally similar dispersion patterns after injection of murexide and fluorescein into flowing buffer solutions. Since, the calibrator solution and metal ion solutions are prepared in the same buffer; the viscosities are same in both solutions.

Table 1
Calculated dispersion coefficients $D_{t,M}$ of metal and the corresponding RSD values using Eq. (3).

Spectrum no.	$D_{t,M}$ (%RSD)	Spectrum no.	$D_{t,M}$ (%RSD)
1	38.19 (0.10)	15	97.05 (0.17)
2	38.50 (0.12)	16	111.28 (0.14)
3	39.43 (0.17)	17	124.89 (0.14)
4	40.63 (0.19)	18	145.99 (0.16)
5	42.24 (0.13)	19	171.63 (0.15)
6	44.54 (0.11)	20	199.30 (0.14)
7	46.92 (0.15)	21	237.76 (0.20)
8	49.74 (0.14)	22	299.50 (0.19)
9	53.43 (0.13)	23	368.73 (0.17)
10	58.00 (0.09)	24	452.94 (0.18)
11	63.18 (0.06)	25	606.20 (0.18)
12	69.88 (0.16)	26	583.77 (0.19)
13	76.69 (0.13)	27	1181.66 (0.20)
14	85.62 (0.15)		

Another factor that affects FI peak shape is the difference in refractive index (RI) between carrier and injected samples. Difference in RI creates a gradient index lens that perturbs the shape of absorbance peaks [52]. In the FI technique presented in here, since the injected samples are prepared in the same carrier solutions, this factor is not important.

Existence of adsorption–desorption processes at the tube walls of the FI manifold also affect the shape of the FI peaks [53]. The tube wall material should not interact with solutes flowing through the system. Dispersion pattern of the dyes murexide and fluorescein were totally similar, proving that no such effects are involved in the studied systems.

Temperature not only affects the formation constant values but is also known to increase D_t values, measured at peak height time, by up to 0.5%/°C [54]. All experiments were performed in a room with controlled temperature within $\pm$ 1.0 °C.

The detector response rate also affects the shape of FI peaks. The response rate of diode array UV–vis spectrophotometer is fast and in this study is not critical, but such a study is critical when detector response rate is slow, for example, when ion selective electrodes (ISEs) are used that besides of having slow response rate, the direction of signal change also affects response [55].

Absorbance values are related to concentrations rather than to activities and the term conditional constant should be used. Activity coefficients were not taken into account by using relatively high concentrated buffer that causes constant ionic strength, so, variations within the concentration gradient did not cause significant uncertainty in the determined β values.

Combination of physical distribution and chemical kinetics creates dispersion in flow injection involving a chemical reaction [56]. For a reliable study of chemical equilibria, the kinetic contribution to

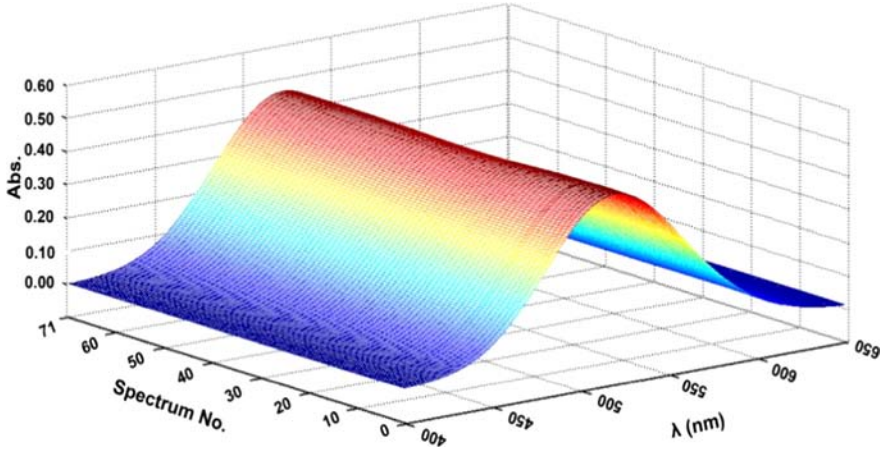

Fig. 4. Spectra obtained from injection of blank solution into ligand solution as carrier. Blank solution: 0.01 mol L^{-1} acetate buffer (50 μl), murexide concentration: 2.23×10^{-4} mol L^{-1} in 0.01 mol L^{-1} acetate buffer, time interval of spectra: 6 S, flow rate: 0.75 ml/min, tube length: 250.0 cm.

the overall dispersion must be minimized. Kinetics has been taken into account by optimizing reaction tube length (250.0 cm) and flow rate (0.75 ml/min). Fig. 3a shows the dispersion pattern when the reaction coil length is 50.0 cm and flow rate is 1.25 ml/min. As can be seen, the changes in dispersion pattern are rather abrupt, especially in the ascending part of the dispersion curve. Calculated dispersion coefficients with this configuration showed the RSD values of (obtained from five repeated subsequent injections) around 25%. These high values of RSD return to the flow turbulency and pulsed flow at high flow rate and also non-equilibrium state in the mixing process of injected sample with carrier when these mixtures reach the flow cell for detection. Fig. 3b shows the dispersion pattern when the reaction coil length and flow rate are 250.0 cm and 0.75 ml/min, respectively. By this configuration, the concentration gradient is monotonic and smooth in both sides of distribution or dispersion peak. The calculated dispersion coefficients from five repeated subsequent injections were in excellent agreement and the precision of the results were good and had RSD of less than 0.2% as shown in Table 1.

3.5. Ligand concentration gradient calculation (C_L)

Having Beer–Lambert law, the total concentration of ligand ($C_{t,L}$) at any time after injection of metal solution can be calculated from injection of blank solution (solution that contains all of the components of metal solution except metal ion) into ligand solution flow. The concentration of ligand at time t after injection ($C_{t,L}$) can be calculated from Eq. (5).

$$C_{t,L} = (A_t/A_0) \times C_{0,L} \quad (5)$$

where, A_0 is the absorbance of ligand at λ_{max} of ligand spectrum, when it is flowing in manifold right before the injected blank solution is reached to the flow cell, A_t is the absorbance (at λ_{max}) of ligand at time t after injected blank solution has reached to the flow cell and $C_{0,L}$ is the initial concentration of ligand. Fig. 4 shows the changes in spectrum of ligand when the blank solution is injected into carrier. Table 2 shows the calculated ligand concentration in the time intervals used for calculation of stability constants after injection of blank solution by Eq. (5), where initial concentration of ligand used as carrier is 2.23×10^{-4} mol L^{-1}, the tube length is 250.0 cm and the flow rate is 0.75 ml/min. As it can be observed from Fig. 4 and Table 2, the changes in ligand concentration are negligible due to high ratio of reaction coil volume to the volume of injected sample. Subsequently, the concentration of ligand at any time step can be assumed constant reasonably and is assumed equal to initial concentration for simplicity.

Table 2
Calculated concentration of ligand (mol L^{-1}) as carrier after injection of blank solution using Eq. (5).

Spectrum no.	$C_{t,L}$	Spectrum no.	$C_{t,L}$
1	0.00021632	15	0.00022024
2	0.00021660	16	0.00022052
3	0.00021688	17	0.00022080
4	0.00021716	18	0.00022108
5	0.00021744	19	0.00022136
6	0.00021772	20	0.00022164
7	0.00021800	21	0.00022192
8	0.00021828	22	0.00022220
9	0.00021856	23	0.00022248
10	0.00021884	24	0.00022276
11	0.00021912	25	0.00022304
12	0.00021940	26	0.00022332
13	0.00021968	27	0.00022360
14	0.00021996		

The other way for taking care of ligand dilution is injecting the mixed metal–ligand solutions, the ligand being at the same concentration as in the carrier, in the flow. In the present study, the calculated formation constants by injecting mixed metal: ligand solution were the same in comparison to those calculated when pure metal solution injected into the ligand solution carrier. Injection of pure metal solution is preferred, because it does not need initial mixing of the reagents before injection and reduced the uncertainty of the calculated final results. We suggest that in the cases that detection is potentiometric [39] or conductometric [57], use mixed metal–ligand solutions for taking care of ligand dilution, as these techniques are very sensitive to small changes in reagents concentration.

3.6. Calculation of stability constants

To show the simplicity, versatility and ease of instrumental setup, the method was applied for the determination of stability constants of murexide as a metallochromic indicator and Cu^{2+}, Cd^{2+}, Pb^{2+}, Ca^{2+} and Co^{2+} in aqueous solution. For taking kinetics into account and also having equilibrated and precise dispersion, the optimum flow rate and tube length was found out to be 0.75 ml/min and 250.0 cm, respectively. However, the initial concentration of injected metal sample must be optimized. The best and repeatable results achieved when the initial concentration of injected metal solutions including Ca^{2+} and Co^{2+}were 0.1 mol L^{-1} and initial concentration of metals including Cu^{2+}, Cd^{2+} and Pb^{2+} were 0.01 mol L^{-1}. These differences are returned to the difference in the stability constants of formed complexes of these metal ions. The larger the equilibrium constant, the higher the concentration of the complex and the lower the required concentration of the reactants. Table 3 shows the stability constants ($\log \beta$) of the complexes calculated by FIA titration, batch titration and the corresponding reported values in the literatures. As can be seen from Table 3, there is a slight differences between results obtained using FIA titration and the reported values in literatures. These differences can be explained by the difference in the experimental conditions, such as pH and ionic strength of the literature and the present study. It is not surprising to see a very good agreement between results of the FIA and batch titration.

Fig. 5a shows the SQUAD output of the estimated concentration of FIA titration of murexide with Cu^{2+}. Other concentration

Table 3
The log β of complexes between murexide and metal ions.

Metal ion	Stoichiometry	FIA titration	Batch titration	literature	Ref. no.
Ca^{2+}	ML	2.550 ($\pm$0.002)	2.540 ($\pm$0.004)	2.690 ($\pm$0.006)	[58,59]
Cd^{2+}	ML	4.270 ($\pm$0.004)	4.190 ($\pm$0.009)	4.15 ($\pm$0.03)	[60]
Co^{2+}	ML	2.570 ($\pm$0.002)	2.510 ($\pm$0.004)	2.48 ($\pm$0.03)	[60,61]
Cu^{2+}	ML	4.350 ($\pm$0.006)	4.28 ($\pm$0.03)	4.3 ($\pm$0.1)	[60–62]
Pb^{2+}	ML	4.500 ($\pm$0.009)	4.54 ($\pm$0.07)	4.40 ($\pm$0.03)	[60]

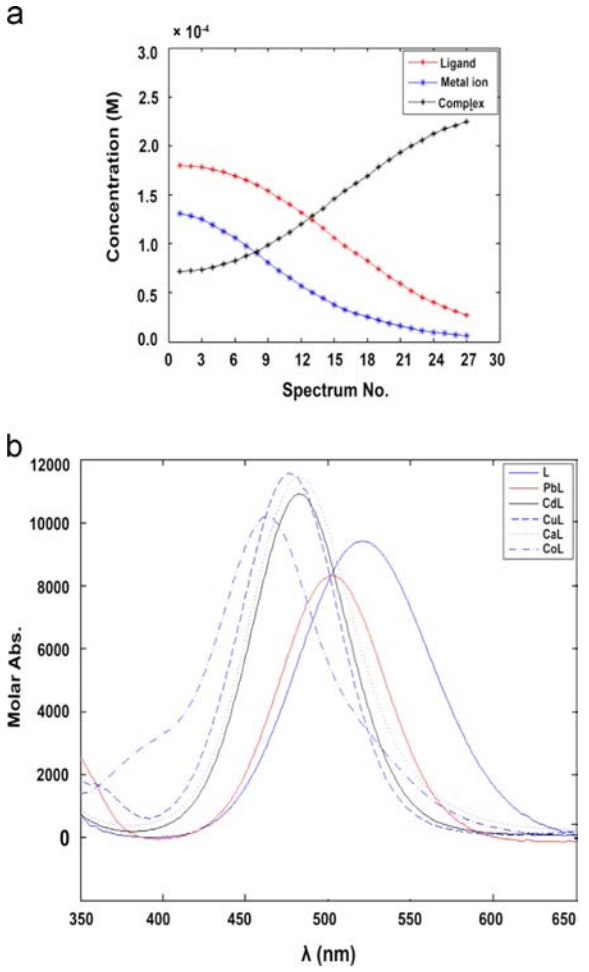

Fig. 5. (a) Estimated concentration profiles of reagent and complex species calculated by SQUAD using murexide–Cu^{2+}data set. (b) Estimated absorption spectra of murexide (L) and 1:1 complex species calculated by SQUAD. The insets of Fig. show the corresponding species.

profiles can be estimated in the same way. Fig. 5b shows the estimated pure absorption spectra of light-absorbing species, free ligand and complexes, obtained by SQUAD computer program using FIA titration datasets of murexide and all studied metal ions.

4. Conclusions

The power of the suggested method is that, after calibration of dispersion pattern, all of the absorption spectral-mole ratio data required by SQUAD computer program to calculate formation constants and estimation of spectral and concentration profiles can be obtained by single injection of metal solution into ligand stream. In addition to the time saving and cost effective due to lower consumption of chemical materials and solvents, the precision of the $\log\beta$ values obtained using FIA titration are higher than the batch titrations which reflect the intrinsic improved precision of automated titration methods in comparison with non-automated titration methods.

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References

[1] C.C. Tang, D. Davalian, P. Huang, R. Breslow, J. Am. Chem. Soc. 100 (1978) 3918–3922.
[2] K. Kumar, C.A. Chang, L.C. Francesconi, D.D. Dischino, M.F. Malley, J. Z. Gougoutas, M.F. Tweedle, Inorg. Chem. 33 (1994) 3567–3575.
[3] A.E. Martell, R.J. Motekaitis, Determination and use of Stability Constants, 2nd edition, Wiley VCH Publishers, Inc., USA, 1992.
[4] J.M. Lehn, Acc. Chem. Res. 11 (1978) 49–57.
[5] R. Bruce King (Ed.), Encylopedia of Inorganic and Bioinorganic Chemistry, John Wiley & Sons, New York, USA, Copyright 1999-2014, http://dx.doi.org/10.1002/9781119951438.
[6] S.E. Castillo-Blum, N. Barba-Behrens, Coord. Chem. Rev. 196 (2000) 3–30.
[7] Y. Sun, C.J. Anderson, T.S. Pajeau, D.E. Reichert, R.D. Hancock, R.J. Motekaitis, A. E. Martell, M.J. Welch, J. Med. Chem. 39 (1996) 458–470.
[8] H. Gampp, M. Maeder, C.J. Meyer, A.D. Zuberbuhler, Talanta 32 (1985) 257–264.
[9] C. Frassineti, L. Alderighi, P. Gans, A. Sabatini, A. Vacca, S. Ghelli, Anal. Bioanal. Chem. 376 (2003) 1041–1052.
[10] M.J. Hynes, J. Chem. Soc., Dalton Trans. 2 (1993) 311–312.
[11] P. Gans, A. Sabatini, A. Vacca, Talanta 43 (1996) 1739–1753.
[12] H. Gampp, M. Maeder, C.J. Meyer, A.D. Zuberbühler, Talanta 32 (1985) 95–101.
[13] D.J. Leggett, Anal. Chem. 49 (1977) 276–281.
[14] L. Sommer, M. Langova, CRC Crit. Rev. Anal. Chem. 19 (1988) 225–269.
[15] H. Abdollahi, F. Nazari, Anal. Chim. Acta 486 (2003) 109–123.
[16] G. Puxty, M. Maeder, K. Hungerbühler, Chemometr. Intell. Lab. 81 (2006) 149–164.
[17] M. Meloun, J. Capek, P. Mikšík, R.G. Brereton, Anal. Chim. Acta 423 (2000) 51–68.
[18] J.J. Kankare, Anal. Chem. 42 (1970) 1322–1326.
[19] H. Gampp, M. Maeder, C.J. Meyer, A.D. Zuberbühler, Talanta 32 (1985) 1133–1139.
[20] J. Ghasemi, A. Niazi, M. Kubista, A. Elbergali, Anal. Chim. Acta 455 (2002) 335–342.
[21] M. Kubista, R. Sjöback, J. Nygren, Anal. Chim. Acta 302 (1995) 121–125.
[22] J. Ghasemi, S. Nayebi, M. Kubista, B. Sjogreen, Talanta 68 (2006) 1201–1214.
[23] M. Meloun, J. Havel, E. Hogfeldt, Computation of Solution Equilibria: A Guide to Methods in Potentiometry, Extraction, and Spectrophotometry, Ellis Horwood, Chichester, UK, ISBN 0-7458-0201-X, 1988.
[24] D.J. Leggett (Ed.), Computational Methods for the Determination of Formation Constants, Vol. 891, Plenum Pub. Corp., New York, USA, 1985.
[25] J. Ghasemi, M. Shamsipur, J. Coord. Chem. 36 (1995) 183–194.
[26] J. Ghasemi, M. Shamsipur, J. Coord. Chem. 31 (1994) 265–272 ⟨http://www.tandfonline.com/doi/abs/10.1080/00958979408024219⟩.
[27] A.S. Meyer Jr., G.H. Ayres, J. Am. Chem. Soc. 79 (1957) 49–53.
[28] E.A.G. Zagatto, C.C. Oliveira, A. Townshend, P.J. Worsfold, Flow Analysis with Spectrophotometric and Luminometric Detection, Elsevier, Amsterdam, 2012.
[29] J. Ruzicka, E.H. Hansen, Anal. Chim. Acta 78 (1975) 145–157.
[30] J. Ruzicka, E.H. Hansen, H. Mosbaek, Anal. Chim. Acta 92 (1977) 235–249.
[31] M. Wójtowicz, J. Kozak, P. Kościelniak, Anal. Chim. Acta 600 (2007) 78–83.
[32] R.S. Vithanage, P.K. Dasgupta, Anal. Chem. 58 (1986) 326–330.
[33] J.F. Tyson, Analyst 112 (1987) 523–526.
[34] J.F. Tyson, Analyst 112 (1987) 527–529.
[35] A.C. Lopes da Conceição, M.L.S.S. Gonçalves, M.M. Correia dos Santos, Anal. Chim. Acta 302 (1995) 97–102.
[36] D.R. Turner, M.M.C. dos Santos, P. Coutinho, M.L. Gonçalves, S. Knox, Anal. Chim. Acta 258 (1992) 259–267.
[37] M.E. Georgiou, C.A. Georgiou, M.A. Koupparis, Anal. Chem. 67 (1995) 114–123.
[38] K. Dong Jo, P.K. Dasgupta, Talanta 60 (2003) 131–137.
[39] M.E. Georgiou, C.A. Georgiou, M.A. Koupparis, Anal. Chem. 71 (1999) 2541–2550.
[40] M.E. Georgiou, M.A. Koupparis, C.A. Georgiou, Analyst 124 (1999) 391–396.
[41] MATLAB Version 7.11.0, Mathworks, Cochituate Place, MA.
[42] M. Kompany-Zareh, Y. Beyad, Anal. Chim. Acta 621 (2008) 163–170.
[43] D.J. Leggett, W.A.E. McBryde, Anal. Chem. 47 (1975) 1065–1070.
[44] J. Ghasemi, H. Peyman, M. Meloun, J. Chem. Eng. Data 52 (2007) 1171–1178.
[45] D.L. Massart, B.G. Vandeginste, L.M.C. Buydens, P.J. Lewi, J. Smeyers-Verbeke, S. De Jong, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier Science Inc., New York, USA, 1997, ISBN: 0444897240.
[46] A.K. Smilde, R. Tauler, J. Saurina, R. Bro, Anal. Chim. Acta 398 (1999) 237–251.
[47] J.T. Vanderslice, A.G. Rosenfeld, G.R. Beecher, Anal. Chim. Acta 179 (1986) 119–129.

[48] D. Betteridge, W.C. Cheng, E.L. Dagless, P. David, T.B. Goad, D.R. Deans, D. A. Newton, T.B. Pierce, Analyst 108 (1983) 17–32.
[49] G. Gerhard, R.N. Adams, Anal. Chem. 54 (1982) 2618–2620.
[50] V. Murugaiah, R.E. Synovec, Anal. Chim. Acta 246 (1991) 241–249.
[51] R. Sitaraman, S.H. Ibrahim, N.R. Kuloor, J. Chem. Eng. Data 8 (1963) 198–201.
[52] R.A. Leach, J. Ruzicka, J.M. Harris, Anal. Chem. 55 (1983) 1669–1673.
[53] S.S. Vives, M.J.M. Hernández, J.L.M. Herrera, G.R. Ramos, Anal. Chim. Acta 268 (1992) 29–38.
[54] C.L.M. Stults, A.P. Wade, S.R. Crouch, Anal. Chim. Acta 192 (1987) 301–308.
[55] D.R. Turner, S. Knox, M. Whittieid, M. dos Santos, C. Pescada, M.D. Goncalves, Anal. Chim. Acta 226 (1989) 239–246.
[56] J.M. Hungerford, G.D. Christian, Anal. Chim. Acta 200 (1987) 1–19.
[57] M. Joshaghani, M.B. Gholivand, F. Ahmadi, Spectrochim. Acta Mol. Biomol. Spectrosc. 70 (2008) 1073–1078.
[58] S. Kashanian, M.B. Gholivand, S. Madaeni, A. Nikrahi, M. Shamsipur, Polyhedron 7 (1988) 1227–1230.
[59] K.S. Balaji, S.D. Kumar, P. Gupta-Bhaya, Anal. Chem. 50 (1978) 1972–1975.
[60] M. Shamsipur, N. Alizadeh, Talanta 39 (1992) 1209–1212.
[61] M. Shamsipur, A. Esmaili., M.K. Amini, Talanta 36 (1989) 1300–1302.
[62] F. Famoori, S. Haghgoo, M. Shamsipur, Talanta 37 (1990) 1107–1108.